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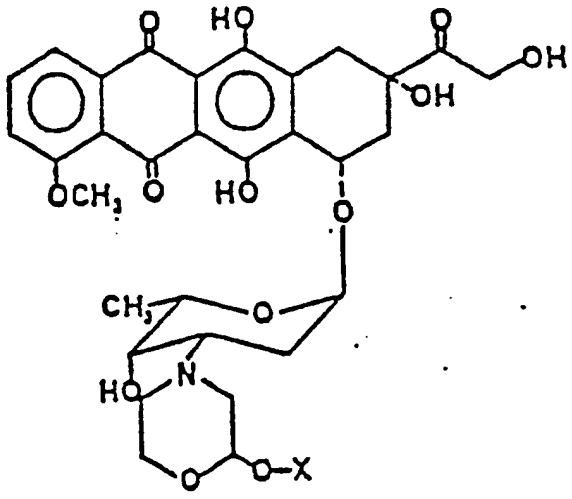
(54) **Morpholinyl derivatives of doxorubicin and process for their preparation.**

(57) Morpholinyl derivatives of doxorubicin having the general formula A:



A

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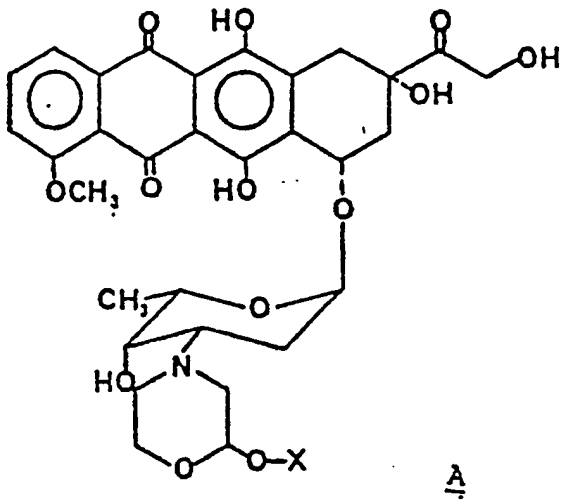
configuration at carbon atom C-2" of the morpholino ring, are antitumor agents.

MORPHOLINYL DERIVATIVES OF DOXORUBICIN AND PROCESS FOR THEIR PREPARATION

The invention relates to anthracycline glycosides, to processes for their preparation and to pharmaceutical compositions containing them.

The invention provides new anthracycline glycosides of general formula A in which the 3'-nitrogen atom is enclosed in a 2-alkoxy-4-morpholinyl ring:



A

wherein X represents a linear or branched C_1 -C_6 alkyl group or a benzyl residue -$CH_2C_6H_5$, and pharmaceutically acceptable acid addition salts thereof. The preferred salt is the hydrochloride salt.

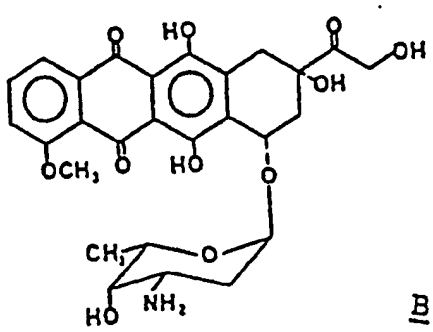
Morpholino-anthracyclines are well known compounds endowed with promising antitumor activity on experimental murine tumors [see: E.W. Acton in Bioactive Molecules, 55-101, vol 6, Edited by J.W. Lown, Elveiser 1988]. Among these, 2-methoxy-4-morpholinyl anthracyclines (X = OCH_3) have been already claimed in our patent US-A-4,672,075. These compounds were prepared through a reductive-alkylating process using a chiral-dihaldehyde. In the present invention, on the other hand, the substituted morpholinyl rings are prepared through bis-alkylation of the 3'-amino group of anthracyclines with novel chiral 1,5-diiodo-2-alkoxy or -benzyloxy derivatives that are within the scope of the invention.

The preferred anthracycline glycosides of general formula A include:

A1: 3'-deamino-3'-(2"(S)-benzyloxy-4"-morpholinyl)-doxorubicin (X = $CH_2C_6H_5$),
A2: 3'-deamino-3'-(2"(S)-ethoxy-4"-morpholinyl)-doxorubicin (X = C_2H_5),
A3: 3'-deamino-3'-(2"(R)-isopropyloxy-4"-morpholinyl)-doxorubicin (X = $CH(CH_3)_2$),
A4: 3'-deamino-3'-(2"(S)-methoxy-4"-morpholinyl)-doxorubicin (X = CH_3)
A5: 3'-deamino-3'-(2"(R)-methoxy-4"-morpholinyl)-doxorubicin (X = CH_3)

and their hydrochloride salts. The compounds may have a (S) or (R) configuration at carbon atom C-2" of the morpholino ring.

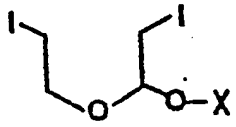
The new anthracycline glycoside antibiotics of the invention, i.e. those of general formula A, are prepared by the formation of a substituted morpholinyl ring at C-3' on the sugar moiety of the antitumor anthracycline glycoside doxorubicin (B):



B

The present invention therefore provides a process for the preparation of an anthracycline glycoside of formula A or a pharmaceutically acceptable acid addition salt thereof, which process comprises:

(i) reacting doxorubicin or an acid addition salt thereof, for example the hydrochloride salt, with a diiodo compound of general formula C:



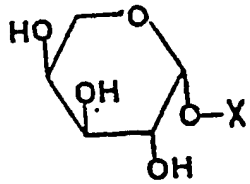
C

wherein X is as defined above; and

(ii) if desired, converting the anthracycline glycoside of formula A thus obtained into a pharmaceutically acceptable acid addition salt thereof.

The alkylation of the C-3' amino group of doxorubicin or the doxorubicin salt is typically performed in step (i) in a polar aprotic solvent and in the presence of a dry organic base such as triethylamine. Reaction is generally carried out at room temperature from eight to twenty four hours. The carbon atom C-2 bearing the -OX group in the diiodo compound may have a (S) or (R) configuration. In a preferred embodiment, doxorubicin or its hydrochloride, dissolved in a polar aprotic solvent is reacted, at room temperature and in the presence of a dry organic base, with the diiodo compound of general formula C to give the corresponding morpholinyl doxorubicin derivative of formula A which, after purification on a silica gel column using as eluting system methylene chloride-methanol (97:5 v/v), is isolated, by treatment with methanolic anhydrous hydrogen chloride, as its hydrochloride.

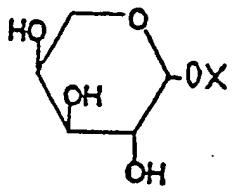
The invention also provides a process for the preparation of optically pure diiodo compounds C, starting from sugar precursors such as the compound of general formula S derived from L-arabinose:



S

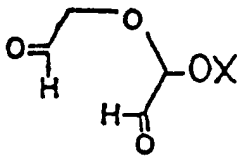
wherein X is as defined above. This process comprises:

(a) subjecting to periodate oxidation a compound of formula S :



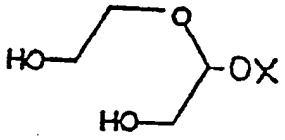
wherein X is as defined above;

(b) reducing the thus-obtained dialdehyde derivative of formula D :



wherein X is as defined above;

(c) sulfonating the thus-obtained dihydroxy derivative of formula E :



wherein X is as defined above; and

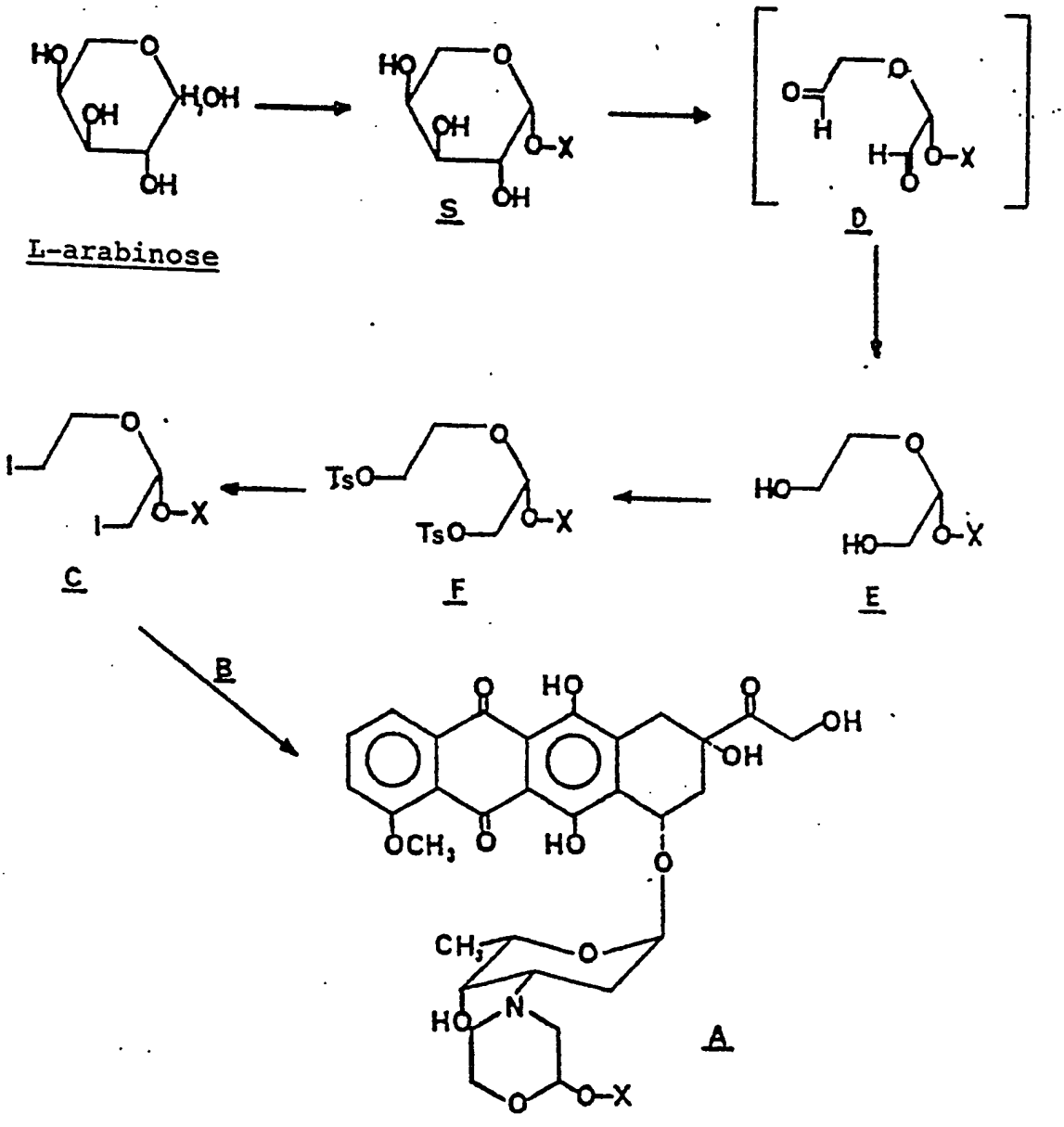
(d) iodinating the sulfonated derivative thus obtained.

In order to prepare the diiodo compounds C, 1-substituted sugars S , prepared following standard procedures described in "Methods on Carbohydrate Chemistry" Acad. Press., Vol 1, (1962), are first transformed into dialdehyde derivatives D . Generally, D- or L-arabinose is employed as a starting material. This is reacted with an alcohol X-OH thereby to form the compound of formula S . The dialdehyde derivatives can be obtained by using periodate oxidation in water, then reduced to 1,5-dihydroxy-2-alkoxy or -benzyloxy-3-oxa-pentane E by using reducing agents such as sodium borohydride or sodium cyanoborohydride at pH 6.5 in a mixture of water and methanol.

The resultant dihydro compounds E are sulfonated at the 1- and 5-hydroxyl groups, typically by using p-toluensulfonyl chloride in pyridine at 4 °C to give the sulfonyl esters of formula F from which the diiodo derivatives C are obtained upon treatment with sodium or potassium iodide in aprotic solvent such as methylethylketone at 85 °C from one to two days. The sequence of these reactions do not affect the chirality at C-2 of the diiodo derivatives C which is the same of the starting sugars S.

A preferred embodiment of a process according to the invention is illustrated by the following reaction Scheme:

Reaction Scheme



Ts=O2 S-C6 H4 pCH3

The present invention also provides pharmaceutical compositions comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, an anthracycline glycoside of formula A or a pharmaceutically acceptable acid addition salt thereof. Conventional carriers or diluents may be used. The composition may be formulated and administered, for example intravenously, in conventional manner.

The anthracycline glycosides of formula A and pharmaceutically acceptable acid addition salts thereof are antitumour agents. They may be used to treat a patient with a tumour by administration of a therapeutically effective amount thereof. The compounds can be used to inhibit the growth of a tumour and are non-toxic at therapeutic doses.

The following Examples illustrate the invention.

Example 1

Preparation of 1,5-di(p-toluensulphonyl)oxy-2(s)-benzyloxy-3-oxa-pentane (F1)

L-arabinose (3 g, 0.022 mole) and benzyl alcohol (15 ml) were heated under stirring in presence of Dowex 50Wx2 (2 g) in acidic form. After four hours, the mixture was cooled and filtered. The solvent was removed under reduced pressure and 1-benzyl-β-L-arabinopyranoside (S1, 3.5 g) was recovered from acetone. TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride/methanol/water (120/20/2 by volume) Rur: Rf = 0.47. $[\alpha]_D = +215^\circ$ (c = 1% water).

1-benzyl-β-L-arabinopyranoside (S1, 3.48 g; 0.0145 mole) was dissolved in water (100 ml) and treated with sodium periodate (5.6 g, 0.026 mole) at 0° C for two hours. Then barium chloride was added and the mixture was brought to pH 7 with barium carbonate, filtered off and washed with water. The aqueous solution was concentrated under reduced pressure to a syrup and extracted with acetonitrile (50 ml). The organic phase was diluted with a mixture of methanol (20 ml) and water (10 ml) and treated with sodium cyanoborohydride (0.3 g) dissolved in water (5 ml). After 15 minutes the mixture was brought to pH 7 by adding Dowex 50Wx2 and filtered. The solvents were removed under reduced pressure to give 1,5-dihydroxy-2(S)-benzyloxy-3-oxa-pentane (E1, 2.6g, yield 85%). TLC on Kieselgel Plate-F_{254} (Merck), eluting system methylene chloride/methanol (10/1 by volume) Rur: Rf = 0.28. Compound E1 (2.6 g) was dissolved in dry pyridine and added with p-toluensulfonylchloride (6.67 9). The mixture was kept at 0° C overnight, then poured into ice-water and extracted with methylene chloride. The organic phase was washed with water, separated off, dryied over anhydrous sodium sulphate and filtered off. The solvent was removed under reduced pressure to afford 1,5-di(p-toluensulphonyl)oxy-2(S)-benzyloxy 3-oxa-pentane (F1, 4.3 g, yield 68%). TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride/acetone (98/2 by volume) $R_f = 0.55$

^{1}HNMR ($CDCl_3$, 400 MHz) δ:

2.42 (s, 6H, two CH_3Ts); 3.65, 3.69 (two dt, J = 4.7, 11.7Hz, 2H, TsOCH,CH_2O); 3.94 (two, dd, J = 5.3, 10.5Hz, 2H, OCH-CH_2-OTs); 4.08 (t, J = 4.7Hz, 2H, Ts-O-CH_2CH_2-O); 4.46, 4.56 (two, d, J = 11.7Hz, 2H, OCH_2-Ph); 4.72 (t, J = 5.3Hz, 1H, O-CH-CH_2-OTs); 7.2-7.8 (m, 13H, aromatics).

Example 2

Preparation of 1,5-diiodo-2(S)-benzyloxy-3-oxa-pentane (C1).

Compound F1 (4.3 g, 8.3 mmole), prepared as described in Example 1, was dissolved in methylethylketone (50 ml) and added with sodium iodide (7.4 g, 49 mmole). The mixture was kept at 95° C for twentyfour hours. After that, the solvent was removed under reduced pressure and the residue was extracted with n-hexane. The organic phase was concentrated to a syrup to give the title compound C1 (3.5 g, yield 90%). TIC on Kieselgel Plate F_{254} (Merck), eluting system n-hexane/ ethyl acetate (10/0.5 by volume) $R_f = 0.34$

^{1}HNMR ($CDCl_3$, 400 MHz) δ :

3.27 (t, J = 6.8Hz, 2H, J-CH_2CH_2-O); 3.30 (d, J = 5.5Hz, 2H, O-CH-CH_2-J); 3.84 (m, 2H, J-CH_2,CH_2-O); 4.63, 4.74 (two d, J = 11.7Hz, 2H, O-CH_2Ph); 4.81 (t, J = 5.5Hz, 1H, O-CH-CH_2-J); 7.3-7.5 (m, 5H, aromatics).

Example 3

Preparation of 3′-deamino-3′[2(S)-benzyloxy-4-morpholinyl] doxorubicin (A1)

To a solution of doxorubicin hydrochloride (0.5 g, 0.86 mmole) in dry dimethylformamide (20 ml) was added 1,5-diiodo-2(S)-benzyloxy-3-oxa-pentane (C1, 3.5 g, 7.54 mmole) and dry triethylamine (3.6 ml, 2.6 mmole). The mixture was kept at room temperature for 36 hours, then was poured in water and extracted with methylene chloride. After standard work-up, the crude product was purified on silicic acid column using as eluting system a mixture of methylene chloride/methanol (97/5 by volume), to give, after treatment with methanolic anhydrous hydrogen chloride, the title compound A1 (0.3 g, yield 46%) as hydrochloride salt.
TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride/methanol (10/1 by volume) $R_f = 0.6$.
FD-MS: m/e 756 (M+)
^{1}HNMR of free base ($CDCl_3$, 200 MHz) δ:
1:37 (d, J = 6.6Hz, 3H, 5'-CH_3); 1.76 (m, 2H, 2'-CH_2); 2.14 (dd, J = 3g, 14.8Hz, 1H, 8ax-H); 2.2-2.7 (m, 6H, CH_2-N-CH_2, 8eq-H, 3'-H); 3.00 (d, J = 18.8Hz, 1H, 10ax-H); 3.55, 4.00 (two m, 2N, O-CH_2CH_2N); 3.68 (s, 1H, 4'-H); 3.94 (q, J = 6.6Hz, 1H, 5'-H; 4.08 (s, 3H, OCH_3); 4.51, 4.77(two d, J = 12.1Hz, 2H, OCH_2Ph); 4.65 (dd, J = 2.6, 4.0Hz, 1H, OCH(OCH_2Ph)CH_2N); 4.71 (s, 1H, $COCH_2$OH); 5.28 (dd, J = 2.4, 3.8Hz, 1H, 7-H); 5.54 (m, 1H, 1'-H); 7.2-8.1 (m, 8H, aromatic H's); 13.22 (s, 1H, 11-OH); 13.95 (s, 1H, 6-OH).

Example 4

Preparation of 1,5-diiodo-2(S)-ethoxy-3-oxa-pentane (C2).

The title compound C2 was prepared starting from L-arabinose (3 g) following sequential reactions as described in Example 1 and Example 2.
1-ethyl-α-L-arabinopyranoside (S2): $[\alpha]_D = +233.5^\circ$ (c = 1% water)
1,5-dihydroxy-2(S)-ethoxy-3-oxa-pentane (E2): TLC on Kieselgel Plate F_{254} (Merck) eluting system methylene chloride/methanol (10/1 by volume) $R_f = 0.33$.
1,5-di(p-toluensulphonyl)oxy-2(S)-ethoxy-3-oxa-pentane (F2):
TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride/acetone (98/2 by volume) $R_f = 0.56$
^{1}HNMR ($CDCl_3$, 200MHz) δ:
1.11 (t, J = 7.0Hz, 3H, OCH_2CH_3); 2.43 (s, 6H, two CH_3-Ts), 3.44, 3.58 (two dq, J = 7.0, 9.4Hz, 2H, OCH_2CH_3,); 3.68 (m, 2H, O-CH_2-CH_2-OTs); 3.89 (d, J = 5.4, 2H, o-CH-CH_2-OTs); 4.10 (t, J = 4.8Hz, 2H, OCH_2-CH_2OTs); 4.61 (t, J = 5.4Hz, 1H, O-CH-CH_2OTs); 7.3-7.8 (m, 8H, aromatic H's).
1,5-diiodo-2(S)-ethoxy-3-oxa-pentane (C2):
TLC on Kieselgel Plate F_{254} (Merck), eluting system n-hexane/ethyl acetate (10/0.5 by volume) $R_f = 0.37$
^{1}HNMR ($CDCl_3$,, 200MHz) δ:
1.24 (t, 7.0Hz, 3N, OCH_2CH_3); 3.23 (d, J = 5.6Hz, 2H, J-CH_2CH-O); 3.27 (t, J = 6.8Hz, 2H, J-CH_2CH_2-O); 3.58, 3.77 (two dq, J = 7.0, 9.3Hz, 2H, OCH_2CH_3); 3.78, 3.87 (two dt, J = 6.8, 10.6Hz, 2H, J-CH_2CH_2-O); 4.70 (t, J = 5.6Hz, 1H, O-CH-CH_2-J).

Example 5

Preparation of 3'-deamino-3'[2(S)-ethoxy-4-morpholinyl] doxorubicin (A2)

Doxorubicin hydrochloride (0.5 g) was reacted with compound C2 (3 g) following the same procedure reported in Example 3 to give the title compound A2 (0.28 g) as hydrochloride salt.
TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride/methanol (10/1 by volume) $R_f = 0.58$
FD-MS: m/e 694 (M+)
^{1}HNMR as free base ($CDCl_3$, 200MHz) δ:
1.16 (t,J = 7.0Hz, 3H, OCH_2,CH_3); 1.36 (d, J = 6.4Hz, 3H, 5'CH_3); 1.7-1.8 (m, 2H, 2'-CH_2,); 2.16 (dd, J = 4.1, 14.7Hz, 1H, 8ax-H); 2.3-2.6 (m, 6H, CH_2,-N-CH_2);, 8eq-H, 3'-H); 3.02 (d, J = 18.8Hz, 1H, 10ax-H); 3.26 (dd, 1.9, 18.8Hz, 1H, 10eq-H); 3.46, 3.75 (two dq, J = 7.0, 9.8Hz, 2H, OCH_2CH_3); 3.53, 3.93 (two m, 2H, OCH_2CH_2,N); 3.68 (s, 1H, 4'-H); 3.93 (q, J = 6.4Hz, 1H, 5'-H); 4.08 (s, 3H, OCH_3); 4.56 (dd, J = 2.3, 4.7Hz, 1H, OCH(OCH_2CH_3)CH_2N); 4.72 (s, 1H, 9-OH); 4.74 (s, 2H, $COCH_2$OH); 5.30 (m, 1H, 7-H); 5.55 (m, 1H, 1'-H); 7.3-8.1 (m, 3H, aromatic H's); 13.25 (s, 1H, 11-OH); 13.97 (s, 1H, 6-OH)

Example 6

Preparation of 1-5-diiodo-2(R)-isopropyloxy-3-oxa-pentane (C3)

The title compound C3 was prepared starting from L-arabinose (3 g) following sequential reactions as described in Example 1 and Example 2.

1-isopropyl-β-L-arabinopyranoside (S3): $[\alpha]_D = +225^\circ$ (water)

1,5-dihydroxy-2(R)-isopropyloxy-3-oxa-pentane (E3):

TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride/methanol (10/1 by volume) $R_f = 0.36$

1,5-di(p-toluensulphonyl)oxy-2(R)-isopropyloxy-3-oxa-pentane (F3)

TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride/acetone (95/2 by volume) $R_f = 0.55$

^{1}HNMR ($CDCl_3$, 200MHz) δ:

1.05, 1.10 (two d, J = 6.2Hz, 6H, $CH(CH_3)_2$); 2.42 two s, 6H, CH_3-Ts); 3.64 (two m, 2H, Ts-O-CH_2CH_2-O); 3.76 (m, 1H, $CH(CH_3)_2$); 3.84 (m, 2H, O-CH-CH_2-OTs); 4.08 (t, J = 5.6Hz, 2H, Ts-O-CH_2CH_2-O); 7.3-7.8 (m, 8H, aromatic H's).

1,5-diiodo-2(R)-isopropyloxy-3-oxa-pentane (C3)

TLC on Kieselgel Plate F_{254} (Merck), eluting system n-hexane/ethyl acetate (10/0.5 by volume) $R_f = 0.40$

^{1}HNMR ($CDCl_3$, 200MHz) δ:

1.20, 1.22 (two d, J = 6.4Hz, 6H, $CH(CH_3)_2$); 3.24 (d, J = 5.6Hz, 2H, O-CH-CH_2-J); 3.28 (t, J = 6.7Hz, 2H, J-CH_2CH_2-O); 3.6-3.8 (m, 2H, J-CH_2CH_2-O); 3.94 (m, 1H, $CH(CH_3)_2$); 4.76 (t, J = 5.6Hz, 1H, O-CH-CH_2-J).

Example 7

Preparation of 3'-deamino-3-[2(R)-isopropyloxyl-4-morpholinyl] doxorubicin (A3)

Doxorubicin hydrochloride (0.5 g) was reacted with compound C3 (3.2 g) following the same procedure reported in Example 3 to give the title compound A3 (0.21 g) as hydrochloride salt.

TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride/methanol (10/1 by volume) $R_f = 0.55$

FD-MS: m/e 708 (M+)

^{1}HNMR as free base ($CDCl_3$, 200MHz) δ:

1.09, 1.16 (two d, J = 6.0Hz, 6H, $CH(CH_3)_2$); 1.36 (d, J = 6.6Hz, 3H, 5'-CH_3); 1.80 (m, 2H, 2'CH_2); 2.15 (dd, J = 4.0, 14.9Hz, 1H, 8ax-H); 2.3-2.8 (m, 6H, CH_2NCH_2, 8eq-H, 3'-H); 2.97 (d, J = 18.8Hz, 1H, 10ax-H); 3.26 (d, J = 18.8Hz, 1H, 10eq-H); 3.54 (m, 1H, O-CH(H)CH_2N); 3.74 (s, 1H, 4'-H); 3.81-4.1 (m, 3H, OCH(H)CH_2N, 5'-H, $OCH(CH_3)_2$); 4.08 (s, 3H, OCH_3); 4.66 (s, 1H, 9-OH); 4.68 (dd, J = 2.2, 4.9Hz, 1H, OCH[$OCH(CH_3)_2$]-CH_2N); 4.75 (s, 2H, $COCH_2OH$); 5.28 (m, 1H, 7-H); 5.55 (m, 1H, 1'-H); 7.3-7.8 (m, 3H, aromatic H's); 13.24 (s, 1H, 11-OH); 13.97 (s, 1H, 6-OH).

Example 8

1,5-dihydroxy-2(S)-methoxy-3-oxa-pentane (E4)

1,5-dioxo-2(S)-methoxy-3-oxa-pentane (D4) (1.5 g, 11mmole), prepared as described in "Methods on Carbohydrate Chemistry" Acad.Press., Vol.1, 445, (1962), was dissolved in a mixture of water (10 ml) and methanol (10 ml) and treated with sodiumborohydride (0.1 g) dissolved in water (2 ml). After 20 minutes the solution was brought to pH 7 with an acidic resin Dowex 50WX2, filtered off and the solvent was removed under reduced pressure to give 1.4 g (Yield 93%) of the title compound TLC on Kieselgel Plate F_{254} - (Merck), eluting system methylene chloride:methanol (10:1 by volume) brown spot at $R_f = 0.24$ after heating the TLC plate previously sprayed with sulforic acid.

^{1}HNMR (200 MHz, DMSO-d_6) δ:

3.26 (S, 3H, OCH_3); 3.4-3.6 (m, 6H, -CH_2-CH_2-O-CH-CH_2-); 4.37 (t, J = 5.4 Hz, 1H, O-CH-O); 4.40 (bm, 2H, HO-CH_2CH_2, CH_2CH_2-OH)

Example 9

1,5-di(p-toluensulfonyl)oxy-2(S)-methoxy-3-oxa-pentane (F4)

1,5-dihydroxy-2(S)-methoxy-3-oxa-pentane (E4) (1.4 g, 10.3 mmole), prepared as described in Example 8, was dissolved in dry pyridine (10 ml) and treated at 0° C with p-toluensulfonylchloride (6.4 g, 0.034 mole). The mixture was kept at 4° C overnight, then was poured into an ice-water mixture and finally extracted with methylene chloride. The organic phase was washed with water, separated off, dried over

anhydrous sodium sulphate, filtered off. The solvent was removed under reduced pressure. The crude material, was chromatographed on a silicic acid column using methylene chloride as eluting agent to give 2.8 g (yield 62%) of pure title derivative,
TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride:acetone (95:5 by volume) brown spot at $R_f = 0.55$ after heating the TLC plate previously sprayed with sulforic acid.
^{1}HNMR (200 MHz, $CDCl_3$) δ:
2.44 (s, 6H, CH_3-Ph); 3.27 (s, 3H, OCH_3); 3.69 (m, 2H, $SO_2OCH_2CH_2$-O); 3.90 (m, 2H, SO_2OCH_2-CH-O); 4.11 (m, 2H, $SO_2OCH_2CH_2$-O); 4.56 (t, J = 5.3Hz, 1H,-O-CH-CH_2); 7.3-7.8 (m, 8H, aromatic H's).

Example 10

Preparation of 1,5-diiodo-2(S)methoxy-3-oxa-pentane (C4)

1,5-di(p-toluensulfonyl)oxy-2(S)-methoxy-3-oxa-pentane (F4) (1.6 g, 3.6 mmole), prepared as described in Example 9, was dissolved in methylethylketone (30 ml) and treated with sodium iodide (3.04 g, 20.2 mmole) at 85° C for two days. After that, the solvent was removed in vacuo and the residue was added with n-hexane (50 ml) and water. The organic phase was separated off, dried over anhydrous sodium sulphate, filtered off. The solvent was removed under reduced pressure to give 1,5-diiodo-2(S)-methoxy-3-oxa-pentane (C4) (1.2 g, yield 86%). TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride, brown spot at $R_f = 0.54$ after heating the TLC plate previously sprayed with sulforic acid.
^{1}HNMR (200 MHz, $CDCl_3$) δ:
3.15 (m, 4H, J-CH_2CH_2-OCH-CH_2-J); 3.40 (s, 3H, OCH_3); 3.80 (m, 2H, J-CH_2CH_2-O); 4.62 (t, J = 5.6 Hz, 1H, O-CH-CH_2).

Example 11

Preparation of 3'-deamino-[2"(S)-methoxy-4"-morpholinyl]-doxorubicin (A4)

To a solution of doxorubicin hydrochloride (80 mg, 0.138 mmole) dissolved in dry dimethylformamide (4 ml) was added 1,5-diiodo-2(S)-methoxy-3-oxa-pentane (C4 , 0.8 g, 2.06 mmole) and triethylamine (0.056 ml, 0.388 mmole). The reaction mixture was kept at room temperature under stirring for 36 hrs, then was poured in water and extracted with methylene chloride. After standard work up, the crude product was purified on silicic acid column using as eluting system amixture of methylene chloride methanol (97.5 : 2.5 by volume), to give, after treatment with methanolic anhydrous hydrogen chloride, 40 mg (yield 45%) of the title compound as hydrochloride salt. TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride:methanol (19:1 by volume) $R_f = 0.15$
Free base: ^{1}HNMR (400 MHz, $CDCl_3$), δ:
13.98 (s, 1H, 6-OH); 13.27 (s, 1H, 11-OH); 8.03 (dd, J = 1.1, 7.7Hz, 1H, 1-H); 7.78 (dd, J = 7.7, 8.6Hz, 1H, 2-H); 7.39 (dd, J = 1.1, 8.6Hz, 1H, 3-H); 5.55 (m, 1H, 1'-H); 5.30 (dd, J = 2.1, 4.1Hz, 1H, 7-H); 4.74 (d, J = 4.5Hz, 14-CH_2OH); 4.74 (S, 1H, 9-OH); 4.49 [dd, J = 2.6, 4.1Hz, 1H, NCH_2-CH(OCH_3)O]; 4.08 (s, 3H, 4-OCH_3); 3.94 (q, J = 6.6Hz, 1H, 5'-H); 3.93 [m, 1H, NCH_2CH(H)O]; 3.67 (m, 1H, 4'-H); 3.54 [m, 1H, NCH_2CH-(H)O]; 3.38 [s, 3H, NCH_2CH-OCH_3]; 3.27 (dd, J = 19, 18.8Hz, 1H, 10-Heq); 3.04 (d, J = 18.8Hz, 1H, 10-Hax); 3.00 (t, J = 4.5Hz, 1H, CH_2OH); 2.60 [dd, J = 4.1, 11.4Hz, 1H, NCH(H)$CHOCH_3$]; 2.6-2.5 (m, 3H, NCH(H)-$CHOCH_3$, NCH_2CH_2O); 2.4-2.3 (m, 2H, 8-Heq, 3'-H); 2.15 (dd, J = 4.1, 14.7Hz, 8-Hax); 1.76 (m, 2H, 2'-CH_2); 1.26 (d, J = 6.6Hz, 3H, 5'-CH_3).

Example 12

Preparation of 1,5-dihydroxy-2(R)-methoxy-3-oxa-pentane (E5)

The title compound was prepared as described in Example 8 starting from 1,5-dioxo-2(R)-methoxy-3-oxa-pentane (D5) which in turn can be prepared as described in "Methods on Carbohydrate Chemistry" Acad.Press., Vol.1, 445, (1962). TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride:methanol, brown spot at $R_f = 0.24$ after heating the TLC plate previously sprayed with sulforic acid.

Example 13

Preparation of 1,5-di(p-toluensulfonyl)oxy-2(R)-methoxy-3-oxa-pentane (F5)

1,5-dihydroxy-2(R)-methoxy-3-oxa-pentane (E5), prepared as describe in Example 12, was transformed into the title compound F5 following the same procedure reported in Example 9. TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride:acetone (95:5 by volume) brown spot at $R_f = 0.55$ after heating the TLC plate previously sprayed with sulforic acid.

Example 14

Preparation of 1,5-diiodo-2-(R)-methoxy-3-oxa-pentane (C5)

1,5-di(p-toluensulfonyl)oxy-2(R)-methoxy-3-oxa-pentane (F5), prepared as described in Example 13, was converted into the diiodo derivative C5 following the procedure described in Example 10. TIC on Kieselgel Plate F_{254} (Merck), eluting methylene chloride, system brown spot at $R_f = 0.54$ after heating the TLC plate previously sprayed with sulfuric acid.

Example 15

Preparation of 3'deamino-[2'(R)-methoxy-4"-morpholinyl]-doxorubicin (A5)

The title compound was prepared by condensing doxorubicin hydrochloride with 1,5-diiodo-2(R)-methoxy-3-oxa-pentane (C5) , prepared as above described, following the procedure reported in Example 11.

TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride:methanol (19:1 by volume) $R_f = 0.13$

Free base: ^{1}HNMR (400 ,MHz, $CDCl_3$) δ:

13;97 (s, 1H, 6-OH); 13.25 (s, 1H, 11-OH); 8.03 (dd, J = 1.1, 7.7Hz, 1H, 1-H); 7.78 (dd, J = 7.6, 7.7Hz, 1H, 2-H); 7.39 (dd, J = 1.1, 7.6Hz, 1H, 3-H); 5.53 (d, J = 3.4Hz, 1H, 1'-H); 5.29 (dd, J = 2.5, 4.1Hz, 1H, 7-H); 4.75 (s, 2H, 14-CH_2OH); 4.71 (S, 1H, 9-OH); 4.46 [dd, J = 2.6, 4.7Hz, 1H, NCH_2-CH(OCH_3)O]; 4.08 (s, 3H, 4-OCH_3); 3.93 (q, J = 6.6Hz, 1H, 5'-H); 3.92 [m, 1H, NCH_2CH(H)O]; 3.70 (m, 1H, 4'-H); 3.56 [m, 1H, NCH_2CH(H)O]; 3.40. [s, 3H, NCH_2CH-OCH_3]; 3.26 (dd, J = 19, 19.9Hz, 1H, 10-Heq); 3.03 (d, J = 19.9Hz, 1H, 10-Hax); 2.66 [dd, J = 2.6, 11.4Hz, 1H, NCH(H)CHOCH_3]; 2.53 (m, 1H, NCH(H)CH_2O); 2.5-2.3 (m, 4H, NCH(H)CH_2O, NCH-(H)CHOCH_3, 8-Heq, 3'H); 2.15 (dd, J = 4.1, 14.7Hz, 8-Hax); 1.8-1.7 (m, 2H, 2'-CH_2); 1.36 (d, J = 6.6Hz, 3H, 5'-CH_3).

BIOLOGICAL ACTIVITY OF 3'-deamino-[2"(S)-methoxy-4"-morpholinyl]doxorubicin (A4) and 3'-deamino-[2"(R)-methoxy-4"-morpholinyl]doxorubicin (A5).

The compounds have been tested in several experimental system in order to ascertain their cytotoxicity and antitumor activity in experimental animals in comparison with parent Doxorubicin. The new anthracyclines result more cytotoxic than the parent drug on LoVo and LoVo Doxorubicin-resistant cell line (LoVo/Dx), Table 1, and are active "in vivo" against doxorubicin-resistant cell lines.

The primary screening "in vivo" was carried out in BDF1 mice bearing P388 Doxorubicin-resistant Johnson's leukemia (P388/Dx) (10^5 cell/mouse). The drugs were administered iv on day 1 after tumor inoculation. Results are reported in Table 2. Both compounds were active and more potent than Doxorubicin.

Compound A4 has also been tested on Doxorubicin-resistant P388 Schabel "in vitro", Table 3, and "in vivo" on BDF1 mice (10^6 cell/mouse), treatmcnt iv on day 1 after the tumor inoculation, Table 4. Finally, compound Ia has been studied on solid tumor such as mammary murine and human carcinoma (MX1) with iv and oral route, Table 5 and 6.

Table 1

Colony assay test against LoVo and LoVo/Dx resistant cells "in vitro" (treatment for 4 hrs)

Compound	LoVo (IC_{50}=ng/ml)[a]	LoVo/Dx (IC_{50}=ng/ml)[a]	R.I.[b]
Doxorubicin	48.8	2553	52.6
A4	8.7	31.7	3.6
A5	6.5	40.1	6.1

a) IC_{50} = concentration inhibiting 50% of colony growth
b) R.I. = Resistance Index = (IC_{50} LoVo/Dx)/(IC_{50} LoVo)

Table 2

Effect of A4 and A5 on Doxorubicin-resistant P388 Johnson's Leukemia "in vivo".

Compound	O.D.[c] (mg/kg)	T/C[d] %
Doxorubicin	13	86
A4	0.09	250
A5	0.13	244

c) O.D. = Optimal Dose: maximally tollerated dose.
d) T/C% = Median survival time of treated mice over median survival time of controls x 100.

Table 3

Effect of A4 on sensitive and Doxorubicin-resistant P388 Leukemia (Schabel) "in vitro" (treatment 1 hr).

Compound	P388 (IC_{50} ng/ml)[c]	P388/Dx (IC_{50} ng/ml)[c]
Doxorubicin	52.7	4000
A4	7.6	24.3

c) IC_{50} = concentration inhibiting 50% of cellular survival.

Table 4

Effect of A4. on Doxorubicin-resistant P388 Schabel's Leukemia "in vivo".

Compound	O.D.[c] (mg/kg)	T/C[d] %
Doxorubicin	13	100
A4	0.09	153

Table 5

Effect of A4 on murine mammary carcinoma.

Compound	Route and Treatment Schedule	O.D.[a] (mg/kg)	Tumor Inhibition %
Doxorubicin	iv q4dx4[e]	5.85	99
A4	iv q4dx4	0.065	94
	po q4dx4[g]	0.1	97

f) iv q4dx4 = treatment iv every four days for four times
g) po q4dx4 = oral treatment every four days for four times

Table 6

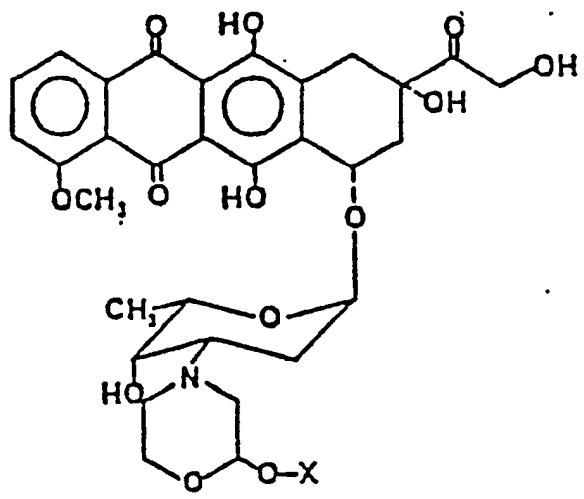
Effect of A4 on mammary human carcinoma (MX1).

Compound	Route and Treatment Schedule	O.D.[a] (mg/kg)	Tumor Inhibition %
Doxorubicin	iv q7dx3[h]	6	72
A4	iv q7dx3	0.05	98
	po q7dx3[i]	0.13	99

h.) iv q7dx3 = treatment iv every seven days for three times
i) po q7dx3 = oral treatment every seven days for three times

Claims

1. An anthracycline glycoside of general formula A:



A

wherein X represents a linear or branched C_1-C_6 alkyl group or a benzyl group, and pharmaceutically acceptable acid addition salts thereof.

2. A compound according to claim 1, which is 3'-deamino-3'-[2"(S)-benzyloxy-4"-morpholinyl]-doxorubicin or its hydrochloride.

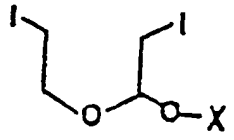
3. A compound according to claim 1, which is 3'-deamino-3'-[2"(S)-ethoxy-4"-morpholinyl]-doxorubicin or its hydrochloride.

4. A compound according to claim 1, which is 3'-deamino-3'-[2"(R)-isopropyloxy-4"-morpholinyl]-doxorubicin or its hydrochloride.

5. A compound according to claim 1, which is 3'-deamino-3'-[2"(S)--methoxy-4"-morpholinyl]-doxorubicin or its hydrochloride.

6. A compound according to claim 1, which is 3'-deamino-3'-[2"(R)--methoxy-4"-morpholinyl]-doxorubicin or its hydrochloride.

7. A process for preparing an anthracycline glycoside of formula A as defined in claim 1, or a pharmaceutically acceptable salt thereof, which process comprises:
(i) reacting doxorubicin or an acid addition salt thereof with a diiodo compound of formula C:



C

wherein X is as defined in claim 1; and
(ii) if desired, converting the anthracycline glycoside of formula A thus obtained into a pharmaceutically acceptable acid addition salt thereof.

8. A process according to claim 7, wherein doxorubicin or its hydrochloride, dissolved in a polar aprotic

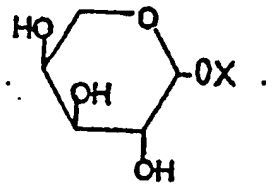
solvent is reacted, at room temperature and in the presence of a dry organic base, with the diiodo compound of general formula C to give the corresponding morpholinyl doxorubicin derivative of formula A which, after purification on a silica gel column using as eluting system methylene chloride-methanol (97:5 v/v), is isolated, by treatment with methanolic anhydrous hydrogen chloride, as its hydrochloride.

9. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, an anthracycline glycoside of formula A as defined in claim 1 or a pharmaceutically acceptable acid addition salt thereof.

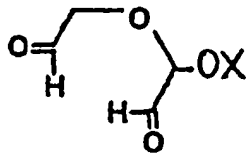
10. An anthracycline glycoside of formula A as defined in claim 1, or a pharmaceutically acceptable acid addition salt thereof, for use as an antitumor agent.

11. A diiodo compound of formula C as defined in claim 7.

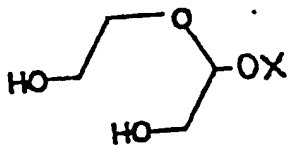
12. A process for the preparation of a diiodo compound of formula C as defined in claim 7, which process comprises:
(a) subjecting to periodate oxidation a compound of formula S :



wherein X is as defined in claim 1;
(b) reducing the thus-obtained dialdehyde derivative of formula D :



wherein X is as defined above;
(c) sulfonating the thus-obtained dihydroxy derivative of formula E :



wherein X is as defined above; and
(d) iodinating the sulfonated derivative thus obtained.

13. A process according to claim 12, wherein D- or L-arabinose is reacted with an alcohol X-OH, wherein X is as defined in claim 1, thereby to form the compound of formula S.

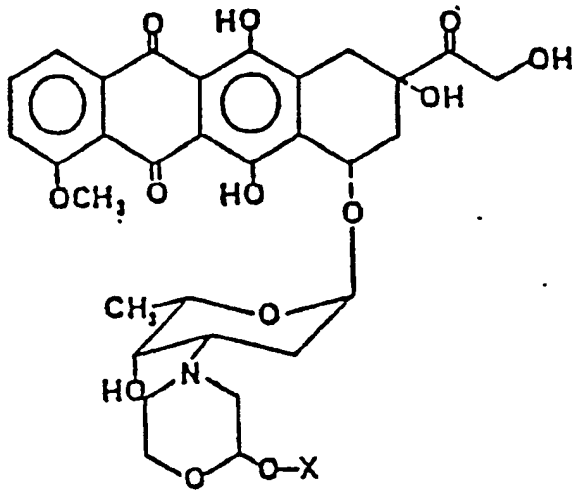
14. A process according to claim 12, wherein the dihydroxy derivative of formula E is reacted with p-toluenesulfonyl chloride in step (c).

15. A process for the preparation of an anthracycline glycoside of formula A as defined in claim 1 or a pharmaceutically acceptable salt thereof, said process being substantially as hereinbefore described in any one of Examples 3, 5,7,11 and 15.

16. A process for the preparation of a diiodo compound of formula C as defined in claim 7, said process being substantially as hereinbefore described in any one of Examples 2, 4, 6, 10 and 14.

Claims for the following Contracting States: ES, GR

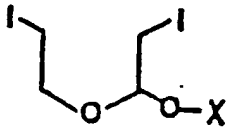
1. A process for preparing an anthracycline glycoside of formula A



A

wherein X represents a linear or branched C_1-C_6 alkyl group or a benzyl group, and pharmaceutically acceptable acid addition salts thereof, which process comprises:

(i) reacting doxorubicin or an acid addition salt thereof with a diiodo compound of formula C:



C

wherein X is as defined in claim 1; and

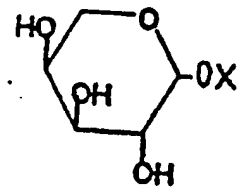
(ii) if desired, converting the anthracycline glycoside of formula A thus obtained into a pharmaceutically acceptable acid addition salt thereof.

2. A process according to claim 1, wherein doxorubicin or its hydrochloride, dissolved in a polar aprotic solvent is reacted, at room temperature and in the presence of a dry organic base, with the diiodo compound of general formula C to give the corresponding morpholinyl doxorubicin derivative of formula A which, after purification on a silica gel column using as eluting system methylene chloride-methanol (97:5 v/v), is isolated, by treatment with methanolic anhydrous hydrogen chloride, as its hydrochloride.

3. A process for the preparation of a diiodo compound of formula C as defined in claim 1, which process

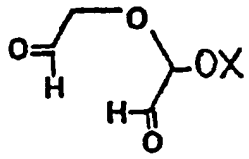
comprises:

(a) subjecting to periodate oxidation a compound of formula S:



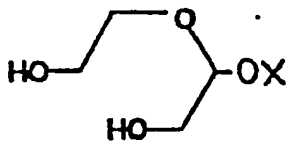
wherein X is as defined in claim 1;

(b) reducing the thus-obtained dialdehyde derivative of formula D:



wherein X is as defined above;

(c) sulfonating the thus-obtained dihydroxy derivative of formula E:



wherein X is as defined above; and

(d) iodinating the sulfonated derivative thus obtained.

4. A process according to claim 3, wherein D- or L-arabinose is reacted with an alcohol X-OH, wherein X is as defined in claim 1, thereby to form the compound of formula S.

5. A process according to claim 3, wherein the dihydroxy derivative of formula E is reacted with p-toluensulfonyl chloride in step (c).

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 12 1905

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X,Y	GB-A-2 172 594 (FARMITALIA) * Whole document *	1-11,15	C 07 H 15/252 A 61 K 31/71
Y	EP-A-0 188 293 (KIRIN) * Abstract; page 22, line 3 - page 23, line 31; page 40, line 5 - page 41, line 3 *	7,8,11,15	C 07 C 43/313
Y	US-A-4 374 980 (H. UMEZAWA) * Whole document *	7,8,11,15	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C 07 H 15/00
A 61 K 31/00
C 07 C 43/00

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	21 March 91	BRENNAN J.

CATEGORY OF CITED DOCUMENTS

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